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associated with the marker. Applicants respectfully traverse this rejection.

At the outset, claim 13, and by dependency claims 14-22, have been amended to recite that the method of the present invention is for detection of antisense oligonucleotides that are 20 to 30 nucleobases in length, uses a probe that comprises a detectable marker and a binding moiety that are covalently bound to the probe, and detects oligonucleotides levels at concentrations between 50 picomolar and 1400 picomolar.

Support for these amendments to the claims can be found throughout the specification as filed. In particular, at page 2, lines 14-20, antisense oligonucleotides having a size range of 20-30 bp are discussed. As a result, it is clear to one of skill that the method of the present invention, when read in the context of the teachings of the instant specification as is required, is a method for detection of antisense oligonucleotides. Further, at page 6, lines 11-16, where the use of the method of the instant invention is described as being for patients on antisense therapy, it becomes even more clear to one of skill in the art that the method of the instant invention is for detection of antisense oligonucleotides. Then, at pages 25-34, the sensitivity and specificity of the assay of the instant invention is described with

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data showing detection of antisense oligonucleotides at levels as low as about 50 picomolar and as high as 2000 picomolar. The method of the present invention is clearly a sensitive method for detection of antisense oligonucleotides in body fluids based on these teachings in the specification as filed. The description of the method as employing a probe that is covalently bound to both a detectable marker and a binding moiety is taught throughout the specification as filed, but in particular at page 3, lines 35 and page 6, lines 23-34.

Impraim et al. (US Patent 6,228,578) describe a non-radioactive hybridization assay and kit for detection of genetic defects, microbial infections or viral infections. In their method, several steps are described. These include first hydrolyzing the RNA in the sample and denaturing the target DNA that is to be detected, next hybridizing the target DNA sequence to a complementary RNA probe to form a double-stranded DNA/RNA hybrid, next capturing the hybrid onto a solid phase where an anti-hybrid antibody has been immobilized, then eliminating non-hybridized probe by digestion with RNase, and finally detecting the bound hybrid. This method does not teach detection of antisense oligonucleotides 20 to 30 nucleobases in length. As is taught in the examples of the Impraim patent, the nucleic acid molecules

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detected are from organisms such as human papilloma virus (HPV) and hepatitis B virus (HBV). Nowhere does this patent exemplify detection of any nucleic acid molecules in the size range of the antisense compounds of the instant invention. As a result, the Impraim assay, as taught in this patent specifically, would not be as sensitive as the assay of the instant invention. This is because the molar sensitivity is dependent on the molecular weight of the entities being detected. In the instant invention, where it is taught that the nucleic acid molecules being detected are antisense compounds (see pages 2 and 6 as listed above for teaching of this size), a sensitivity of as low as about 50 picomolar is much lower than can be achieved for the method of Impraim where the molecular weight of the much larger nucleic acid molecules would result in estimation of a higher sensitivity of the assay (see column 10, lines 43-45). Further, without teaching the actual sensitivity in picomolar concentrations, as is taught in the specification as filed, one of skill would not be able to extrapolate the sensitivity data provided by Impraim for comparability, except to say that with the nucleic acid molecules being much larger, the sensitivity would be inferior to that of the instant method. Additionally, the method of Impraim does not describe in any way a probe that covalently binds both a detectable

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marker and a binding moiety as now claimed. Thus, in these three ways, i.e., use for detection of antisense oligonucleotides, sensitivity of the method, and the type of binding between the probe and the detectable marker and the probe and the binding moiety, the claims as amended distinguish the instant invention from the method of Impraim et al.

MPEP 2131 states that in order to anticipate a claim the reference must teach each and every limitation of the claim. Accordingly, Impraim fails to teach the method of the present invention which recites a method for detection of antisense oligonucleotides and with a sensitivity of between about 50 picomolar and 1400 picomolar, involving use of a probe that has a detectable marker and a binding moiety that are covalently linked to it. Withdrawal of this rejection is therefore respectfully requested.

## II. Rejection of Claims Under 35 U.S.C. 103(a)

Claims 13, 15, 20 and 21 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Tamsamani et al. (1993) in view of Impraim et al. (US Patent 6,228,578). The Examiner suggests it would have been *prima facie* obvious for one of ordinary skill in the art to modify the method of Tamsamani et al. with the method of

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Impraim et al. to teach detection of oligonucleotides by forming hybrids, contacting with a solid support, using a nuclease to degrade non-hybridized probes and then detecting the bound hybrid. Applicants respectfully traverse this rejection.

As discussed *supra* in Section I, the claims as amended recite that the method of the present invention is for detection of antisense oligonucleotides at levels in a liquid sample between about 50 picomolar and 1400 picomolar, wherein both the detectable marker and the binding moiety are bound to probe. Support for these amendments is found throughout the specification as filed but in particular at pages 2, 3, 6 and 24-35.

Temsamani et al. (1993) disclose a method for quantitation and detection of phosphorothioate modified oligonucleotides. The first step in this method is explicitly stated in the abstract and shown in Figure 3 as being immobilization of the oligonucleotide to a solid support, in this case a nylon membrane, before the oligonucleotide is contacted with any type of binding probe. Therefore, an unmodified oligonucleotide is attached. In the present method, the claims recite and the specification teaches that the binding moiety of the probe, not the oligonucleotide itself, is what binds directly to the binding partner which is immobilized to the solid support. Further, Temsamani et al. fail

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to teach, the use of a nuclease of any type to degrade non-hybridized probes. Finally, Tamsamani et al. teach that the detection of oligonucleotides using their assay is at levels only as low as 1.5 nanograms (see page 55, first column and Figure 1). In contrast, the present method detects oligonucleotides at levels as low as about 50 picomolar to levels as high as 2000 picomolar. This range includes the 1400 picomolar level that is claimed in the amended claims. The concentrations between 50 and 1400 picomolar are equivalent to detection of oligonucleotides from 20 to 30 mer at levels between 0.035 and 1.47 nanograms. The lower end of the range as claimed was calculated based on an average molecular weight of a 20 mer oligonucleotide (i.e., MW of 7000 pg/picomole) and a 100 microliter sample size, as taught in the specification as filed. Using these values the calculation was made as follows:

50 picomolar = 50 micromoles/liter

50 picomoles/liter x 7000 pg/picomole x 1 liter/1000 ml  
= 350 pg/ml

In 100 microliters, 350 pg/ml x 0.1 ml = 35 pg = 0.035 ng.

Considering the upper end of the range claimed as 1400 picomolar for a 20 mer oligonucleotide, the same equation can be used with 1400 substituted for 50, resulting in calculation of the upper end of the range at 0.98 ng. If a 30 mer oligonucleotide is considered

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(average MW = 10,500), in a 100 microliter sample, the upper end of the range would be 1.47 picomolar and the lower end would be 0.052 ng. Therefore, the Tamsamani reference does not teach the claimed method which now recites contact of a solid support only after hybridization of the oligonucleotide as well as use of a single-strand specific nuclease to degrade non-hybridized probe, and then detection of antisense oligonucleotides at levels between about 50 and 1400 picomolar.

Impraim et al., as discussed in detail *supra*, teaches a method for detection of nucleic acid segments from DNA of organisms such as bacteria and viruses and detection of genetic defects, microbial infections or viral infections by detecting such pieces of DNA (see abstract of Impraim et al. and *Background of the Invention* section). Therefore, this patent is teaching detection of nucleic acid molecules that are much larger than those of the instant invention, and are not antisense compounds as defined in the specification as filed. Further, the method of Impraim et al. does not specify binding of both double-stranded and single-stranded oligonucleotide moieties to a solid support and does not teach or suggest combining into a probe both the use as a detection device and a binding moiety that are specifically and strongly linked or covalently bound. In the case of Impraim, it is specified that

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binding moieties are monoclonal antibodies specific to the DNA:RNA hybrid and detection is due to use of a chemiluminescent substrate for alkaline phosphatase. Nowhere does Impraim et al. teach or suggest use of a probe that has both binding and detection properties without further steps being added to the method. Nor would one of skill be motivated to add such steps given the different uses of these methods. Finally, the method of Impraim et al. does not have the sensitivity as is now claimed for the instant invention and was discussed in detail *supra*.

To establish a *prima facie* case of obviousness, three basic criteria must be met. MPEP 2143. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art must teach or suggest all claim limitations. Clearly, the combination of prior art cited fails to teach or suggest the limitations of the claims as amended. Neither reference, alone or when combined, would teach or suggest detection of antisense oligonucleotides at concentrations between 50 and 1400 picomolar. This advantage in sensitivity of detection can only be seen with the teachings of the instant specification. Thus, one of



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skill would not have an expectation of success of detection of antisense oligonucleotides at these low levels using the combination of cited art, but only with the teachings of the specification at hand. Accordingly, this combination of cited art fails to establish a *prima facie* case of obviousness as specified under MPEP 2143 and withdrawal of this rejection is respectfully requested.

Claim 14 has been rejected under 35 U.S.C. 103(a) as being unpatentable over Tamsamani et al. and in view of Impraim et al., and further in view of Serres et al. The Examiner suggests that it would have been *prima facie* obvious to one of ordinary skill to combine the oligonucleotide detection method of Tamsamani et al., as modified by Impraim, with the method of Serres et al. for detecting oligonucleotides in plasma. Applicants respectfully traverse this rejection.

As discussed *supra*, the primary reference of Tamsamani et al. (1993), when combined with the reference of Impraim et al., fails to teach the invention of the amended claims. Therefore, adding Serres et al., which teach detection of oligonucleotides in plasma by another, very different method cannot render the instant invention obvious. Clearly, the combination of prior art cited

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fails to teach or suggest the limitations of the claims as amended and withdrawal of this rejection is respectfully requested.

Claims 16 and 17 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Impraim et al., in view of Lind et al. (1998). The Examiner suggests it would have been *prima facie* obvious for one of ordinary skill to combine the oligonucleotide method of Impraim et al. with the Lind et al. method for modification of oligonucleotides with at least one sugar moiety at the 2' position. Applicants respectfully traverse this rejection.

As discussed in detail *supra*, the primary reference of Impraim et al. fails to teach the limitations of the claimed invention which recite antisense oligonucleotide detection at levels as low as 50 picomolar. Therefore, the combination of a secondary reference (Lind et al.) which teaches only methods for modifying oligonucleotides does not render the instant invention obvious. Withdrawal of this rejection is respectfully requested.

Claims 18 and 19 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Impraim et al., in view of Prosnyak et al. (1994). The Examiner suggests that it would have been *prima facie* obvious for one of ordinary skill to combine the oligonucleotide method of Impraim et al. with the Prosnyak et al. method for

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modification of oligonucleotides with at least one 5-methylcytosine moiety. Applicants respectfully traverse this rejection.

As discussed *supra*, Impraim et al. fails to teach the limitations of the amended claims. Therefore, the combination of a secondary reference (Prosnyak et al.) which teaches only methods for modifying oligonucleotides does not render the instant invention obvious. Withdrawal of this rejection is respectfully requested.

Claim 22 has been rejected under 35 U.S.C. 103(a) as being unpatentable over Impraim et al., in view of Lundin et al. (1997). The Examiner suggests it would have been *prima facie* obvious for one of ordinary skill to combine the Impraim detection method with the Lundin S1 nuclease to make the claimed invention. Applicants respectfully disagree with the Examiner's conclusions.

As discussed *supra*, the primary reference of Impraim et al. fails to teach the claimed invention. Therefore, the combination of Lundin et al., which teaches a method for screening large regions of DNA for structural changes, a very different method for a different purpose, cannot render the instant invention obvious. Withdrawal of this rejection is respectfully requested.

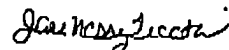
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### III. Conclusion

Applicants believe that the foregoing amendment places this case in condition for allowance. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Respectfully submitted,



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## VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

The claims have been amended as follows:

13. (amended) A method for detecting an antisense oligonucleotide 20 to 30 nucleobases in length in a bodily fluid or extract at concentrations between about 50 picomolar and 1400 picomolar, consisting of the steps of:

~~a) preparing a bodily fluid or extract for analytical detection to form a liquid sample;~~

~~ab) contacting said a liquid sample with a probe complementary to an antisense oligonucleotide that is 20 to 30 nucleobases in length so that the probe and the oligonucleotide can form hybrid moieties in said liquid sample, wherein said probe comprises a detectable marker and a binding moiety and said detectable marker and said binding moiety are covalently bound to said probe;~~

~~bc) placing said liquid sample in contact with a solid support to which a binding partner of said binding moiety is attached so that said hybrid moieties present in said liquid sample will be attached to said solid support, and wherein said binding~~

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partner's ability to detect said antisense oligonucleotide is independent of the sequence of said oligonucleotide;

cd) removing any oligonucleotide from said liquid sample that has not formed a hybrid moiety;

de) contacting said liquid sample with a single strand oligonucleotide-specific nuclease under conditions in which probe which is not hybridized to form said ~~double-stranded oligonucleotide moieties~~ hybrid moieties is degraded and thus is no longer attached to said solid support;

ef) removing any unbound detectable marker from said liquid sample; and

fg) detecting a label associated with said marker wherein the presence of said label indicates the presence of said hybrid moieties bound to said solid support wherein detection of said label at levels above the level characteristic of a liquid sample that was prepared as a blank sample to contain no antisense oligonucleotide indicates the presence of said antisense oligonucleotide in said liquid sample at concentrations between about 50 picomolar and 1400 picomolar.

15. (amended) The method of claims 13, wherein said antisense oligonucleotide comprises at least one phosphorothioate linkage.

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16. (amended) The method of claim 13, wherein said antisense oligonucleotide comprises a modification at the 2' position of at least one sugar moiety.

18. (amended) The method of claim 13, wherein said antisense oligonucleotide comprises at least one modified base.